Chapter 11

The Actomyosin Network and Cellular Motility: A S100A4 Regulatory View into the Process

Stephane R. Gross

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66940

Abstract

Cell migration is a fundamental process responsible for numerous physiological and physiopathological conditions such as inflammation, embryogenesis and cancer. This central aspect of cell biology has seen quantum leaps in our understanding of the coordinated regulations, both spatial and temporal of numerous cytoskeletal proteins and their orchestrations. At the molecular level, this dynamic cellular process can be naively summarised as an engineered cycle composed of three distinct phases of (1) formation of cellular protrusion to initiate contact followed by (2) the adhesion with the external environment/cell-extracellular established connection and (3) the actomyosin force generation to consequently remodel the cytoskeleton. A prominent factor that regulates cellular motility is S100A4, a protein that has received constant attention for its significant role in cellular migration. Consequently, and in order to focus further the impact of this work, the present chapter aims to review some of the actomyosin proteins/complexes that have been demonstrated to be crucial players of the cyclic migration process but are also S100A4 interactors. In doing so, this chapter aims to capture a picture of how expression of this small, calcium-binding protein may, in essence, remodel at different levels the actin organisation and fulfil the motility engineered cycle of protrusion, attachments and contractions.

Keywords: S100A4, Actin, Arp2/3, formin, tropomyosin, myosin, Rho-GTPAses, Rhotekin

1. Introduction

Cellular motility has been an essential cellular phenomenon throughout phylogeny that has allowed organisms to survive, adapt and prosper in different environments. It is engrained in the chemoattraction and nutrient-seeking mechanisms in protozoa such as *Dictyostelium*

discoideum [1]; whilst in metazoan, it is found to be a key concept for physiological regulations during all aspects of life. For instance, cellular migration in the early stages of gastrulation allows the coordinated movements of progenitor cells for the subsequent development of the different layers of precursor tissues and organs [2, 3]. Equally important is the profound effects cellular migration occupies in the process of healing during wound closure and/or tissue regeneration undertaken by tissues of the mesenchyme or epithelium [4]. Cell motility also plays essential functions during all stages of the immune response, from the development of mature effector cells, to endothelium trans-crossing and phagocytosis [5–7]. Given the indispensable roles of cellular migration in these events, and others, it is therefore not surprising to learn that loss of functions of many actin-regulating genes result in embryonic lethality or severe immunodeficiency syndromes [8].

Other than these physiological conditions, cellular motility is essential in regulating some of the physiopathological steps seen in disease. As example, it is well-documented that cellular migration is one of the prominent factors involved in the later stages of carcinogenesis and the subsequent phases of metastasis [9–11]. Cancer cell dissemination is clearly dependent upon the ability of migratory tumour cells to evade away from their initial niche, leading to the colonisation and formation of distant secondary lesions in the body [12].

At the molecular level, cell migration requires the coordinated regulations, both spatial and temporal of numerous cytoskeletal proteins, to orchestrate the dynamic cellular processes needed for cells to acquire movement. In this context, the actin cytoskeleton and the closely linked myosin network play essential functions [13, 14]. The process of cellular motility can be summarised as an engineered cycle composed of three distinct phases which are, (1) formation of cellular protrusion in the forms of lammelipodia and fillopodia to initiate contact and adhesion with the external environment, (2) regulation of cell-extracellular matrix established connections, usually integrin-dependent, and (3) force generation by the actomyosin network which will control both the structure and organisation of the motile architecture [15]. I provide here a brief overview of some of the different elements and protein complexes that are regulated during this migratory cycle, focusing primarily on specific components of the actomyosin complexes.

A group of low-molecular weight polypeptides that has been demonstrated to have key functions in remodelling the overall actin cytoskeletal network is the S100 protein family [16]. Composed of approximately 25 members, the presence of the majority of these in different cellular systems, both in vivo and in vitro has been associated with significant changes in cellular migration. One of the most prominent members of this family to have been linked to regulate cellular motility is S100A4, a protein that has received constant attention for its significant role in promoting cancer metastasis [16–18]. Consequently, and in order to emphasis the impact of this work and strengthened its delivery, I have concentrated our attentions on actomyosin proteins/complexes that have both been demonstrated to be crucial players of the migration process but also S100A4 interactors. In doing so, this chapter aims to capture a picture of how expression of this small, calcium-binding protein may in essence remodel at different levels the actin organisation and fulfil the motility engineered cycle of protrusion, attachments and contractions.

http://dx.doi.org/10.5772/66940

2. The actomyosin machinery in cellular migration

Motility can be seen as a lone activity where a single cell may migrate (also known as amoeboid or mesenchymal migration [19, 20]) or is referred to as collective, if this effort is the result of concerted effort undertaken by numerous cells, either in sheet or clusters [9]. Equally important is the cell physiognomy that will be regulated in the process. Mesenchymal motility as seen during fibroblast migration leads to cellular characteristic of a more elongated spindle-like shape. In this type of migration, an actin-rich leading edge can be observed, where extension of the front leading edge is driven by actin polymerisation [21]. In amoeboid migration, cells adopt a more rounded morphology and rely on the contraction-based membrane blebbing and enriched levels of myosin II at the cell rear [22]. Both of these migratory processes have been shown to play important roles in both physiological and pathological events.

The complexity of the different types of cell migration that can be used is mirrored by the number of different molecular pathways that are available to orchestrate these processes. Among them, however, the remodelling of the actin cytoskeleton and its organisation stands as an irreplaceable circuitry which is undisputably common to all. At the molecular level, this network is considered to provide the platform where physical forces will be exerted. Pushing forces generated by the assembly of filamentous actin (F-actin) will encourage the formation of cellular protrusions, such as filopodia, lamellipodia, blebbing and the most recently characterised invadopodia [23–25]. These changes in actin polymerisation and their dynamics will be directly responsible for reshaping and remodelling the underling plasma membranes.

2.1. Cellular protrusions and regulators

Actin polymerisation. The actin filaments are considered to be the backbone of cellular protrusion, providing the physically necessary special platform that will provide sufficient force to deform the plasma membrane. Their overall organisation relies primarily on their polymerisation from monomeric globular actin (G-actin) into long arrays. This process is regulated by numerous partners but the core regulator lies in ATP hydrolysis to promote actin molecule recognition and bonding between two monomers. When ATP bound G-actin is hydrolysed, the newly created ADP+Pi G-actin structure can form stable filaments. Binding of the nucleotide takes place in the high-affinity binding site located in the deep upper inter-domain cleft of actin (**Figure 1**). The presence of a cleft around exposed subdomains II and IV results in the polarisation of the monomeric structure and is referred to as the pointed end (**Figure 1**). The other exposed side, composed of subdomains I and III is known as the barbed end [26] and constitute the major binding site for most actin binding proteins ([27], **Figure 1**). This is a very important distinction which will result in sticking difference in behavioural characteristics in both G-actin and F-actin, of which polarised polymerisation is only one aspect.

In the early stages of assembly, also known as nucleation, actin protomers aggregate in an energetically unfavourable process to form a dimer that is more likely to dissociate. Addition of another subunit stabilises the complex and represents the nucleus, a state where actin polymerisation is now more favourable than dissociation (**Figure 1**). The association of monomers

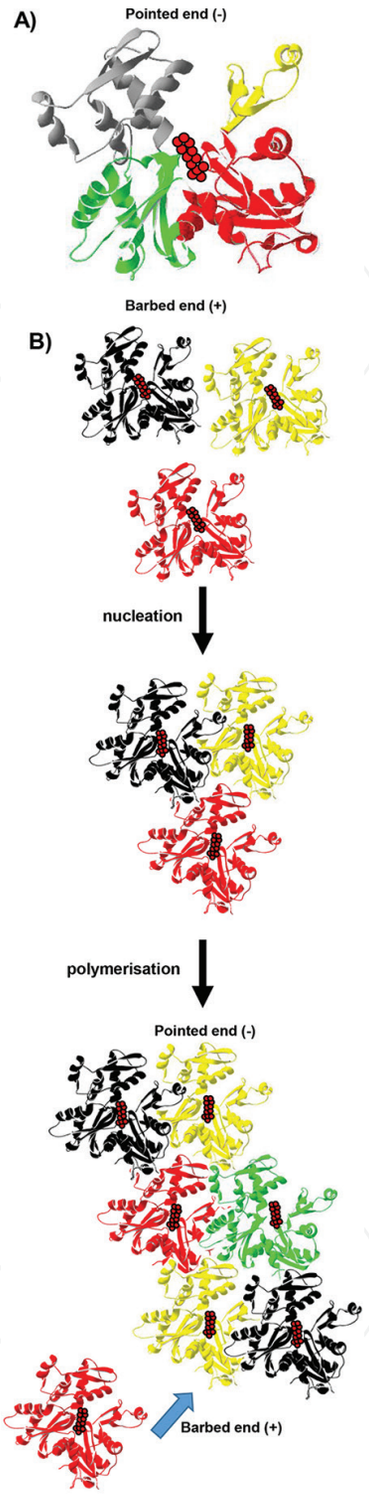

Figure 1. Actin structure and cartoon of F-actin polymerisation. (A) G-actin monomer at 1.54-Å resolution bound to ADP (PDB code 1J6Z) by Otterbein et al. [165] obtained from striated rabbit muscle tissue. Subdomain I (red, residues 1–32, 70–144 and 338–374), subdomain II (yellow, residues 3369), subdomain III (green, residues 145–180 and 270–337) and subdomain IV (grey, residues 181–269) are highlighted, resulting in the orientation of the actin molecule with the pointed end (− end) and the nucleotide cleft in the upper part, and the barbed end (+ end) in the lower part. (B) Process of actin polymerisation highlighting the steps of nucleus formation and filament formation. Please note this is a schematic representation which does not illustrate the current model of actin polymerisation initially proposed by Holmes et al. [166] suggesting that actin filaments are structured as a two right handed long pitch helices of head to tail bound actin subunits or a single left handed short pitch helix with consecutive lateral subunits staggered with respect to another by half a monomer length.

into a trimeric structure is seen as the rate limiting step of the whole polymerisation process as it is reversible where monomers can easily dissociate [28–30]. It is during the stage of nucleation that addition of further actin subunits is supported at both ends. Once the nucleus and newly added monomers have been locked into position by conformational changes, the process of elongation begins and the addition of actin molecules at the barbed end of the filament can be seen, resulting in the formation of structural polarised complexes (**Figure 1**). Whilst G-actin subunits can self-assemble, this process only occurs if the concentration of actin exceeds a critical concentration.

Within cells, a growing number of binding partners, or actin-binding proteins, will act both antagonistically and agonistically to regulate the polymerisation process. Some factors will act as nucleators, such as formins and Arp2/3, facilitating the process through providing a scaffold structure which encourages de novo assembly. Others will regulate the overall structure of filaments through their remodelling in larger structures. Examples provided here will control the cross-linked state of actin filaments through the involvement of bundling regulators such as the tropomyosins and to an extent myosins. Involvements of all these factors, as well as many others that are too numerous to be listed, here, will be responsible for the remodelling of the actin cytoskeleton into different substructures seen during cell migration (**Figure 2**).

When grown in a 2D environment, cells will encourage the formation of differential planar filamentous actin, in the form of filipodia/microvilli or sheet-like structures referred to as lamellipodia (**Figure 2**, [31, 32]). Whilst the former act as sensory organelles that enable cells to probe their local environment, through the formation of thin extensions that are mainly made of long, unbranched bundles, the latter is viewed as the main driving force for locomotion, through the organisation of short branched actin networks (**Figure 2**). In both instances, however, regulation of F-actin polymerisation, especially at their barbed end is essential, in order to control their elongation in the direction of the plasma membrane and is thought to require nucleation-promoting factors where both formins and Arp2/3 have been shown to play key functions (**Figure 2**, [31, 33]).

Formins. The family of formins, encoded by 15 different genes in mammals represent a cluster of large multi-domain proteins, grouped in eight different subfamilies, that regulate actin nucleation and polymerisation, primarily at the barbed end [34, 35]. Their nucleation abilities are regulated by signature regions of the proteins, the formin homology domains 1 and 2 (FH1/FH2), located at the C-terminus (**Figure 3**). Although a clear picture as to how formins nucleate the assembly of actin filament is still under investigation, the C-terminal region has been demonstrated to be a key regulator as it recruits actin monomers in the presence of profilin. The FH2 domain also plays key function during the polymerisation of F-actin as it allows addition of large amounts of actin subunits at the barbed end [36]. This continuous tracking results from alternate contact of the two halves of the FH2 domain with the two most terminal actin subunits in the filament, allowing the sliding of the whole formin molecule through an open/closed conformation as the subunits, remaining bound as subunits are added [37–39]. For some formins, activation is also controlled through the release of the head to tail auto-inhibition as well as through the movement of proteins away from the leading edge [40]. For such formins, classified as diaphanous-related formins (DRF) [41], comprised

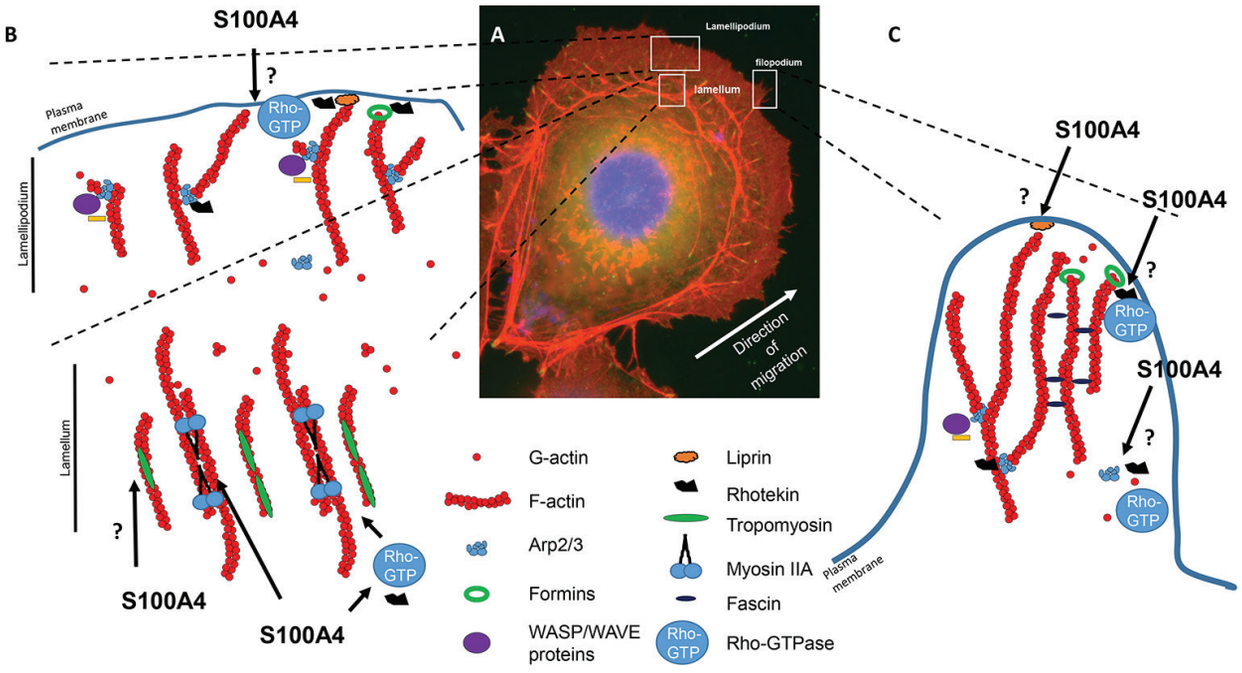

Figure 2. S100A4 interacting actomyosin complexes and their simplified organisation in the different protrusions of a migrating cell. (A) Actin and focal adhesion organization in a HeLa migrating cell. Staining for F-actin using Phalloidin-rhodamin (red) and paxillin with antibodies coupled to FITC (green) in a migrating HeLa cell. In this image, the actin mediated structures of the filopodium and lamellipodium/lamellum are distinctly visible at the leading edge of the cell. B and C present models for the lamellipodium/lamellum and filopodium and the respective molecular organisation within, focusing on the proteins presented in this chapter. (B) A simplified model for lamellipodium/lamellum formation. In the lamellipodium, the Arp2/3 complex via activation by WASP/WAVE complex interacts with actin filaments resulting in the nucleation of new actin filaments from the side of existing filaments. Formin proteins are also found at the barbed end of filaments. Limprin and the Rho-GTPase-Rhotekin complexes could get regulated by S100A4 to promote lamellipodia protrusions. In the lamellum, tropomyosin wrapping around the actin filaments prevents interactions with other actin binding proteins. NMMIIA regulates retrograde flow in the lamellum. At the interface of the lamellipodium–lamellum, actin is depolymerised. Interactions of S100A4 with tropomyosins and the NMMII complexes have been reported and could result in significant changes in their overall organisation. (C) A simplified model for filopodia formation. In this diagram, actin polymerisation promoted by the Arp2/3 complex leads to the branching and extension of nascent individual actin filaments in the filopodium. Recruitment of the formins to this location promotes the elongation of the filaments through the addition of actin monomers at the barbed end. Other actin bundling proteins such as fascin regulates filopodia stability through the clustering of actin filaments. Both the Limprin and the Rho-GTPase-Rhotekin complexes could be regulated by S100A4 to control filopodial protrusions.

of 4 families; diaphanous (Dia including mDia), Dishevelled associated activators of morphogenesis (Daam), formin-like proteins (FMNL) and FH1/FH2 domain-containing proteins (FHOD) in mammals, the auto-inhibitory mechanism relies on the folding of the N-terminal portion, containing domains FH3, which physically obstructs the diaphanous autoregulatory domain (DAD) at the C-terminus and prevents it to interact with actin molecules (**Figure 3**, [42]). Binding of the Rho-GTP to the formin polypeptide in the GBD (GTPase binding domain) region is thought to result, at least in part, to the displacement of the masking DAD region away from the FH3 domain [43]. Molecular mechanisms to explain this process are currently being investigated. The relocalisation of formin to the leading edge is also a key concept to control their activities. Membrane relocalisation has been reported to be performed primarily by Rho-GTPases through their binding to the GBD [44]. Other studies have also revealed that

http://dx.doi.org/10.5772/66940

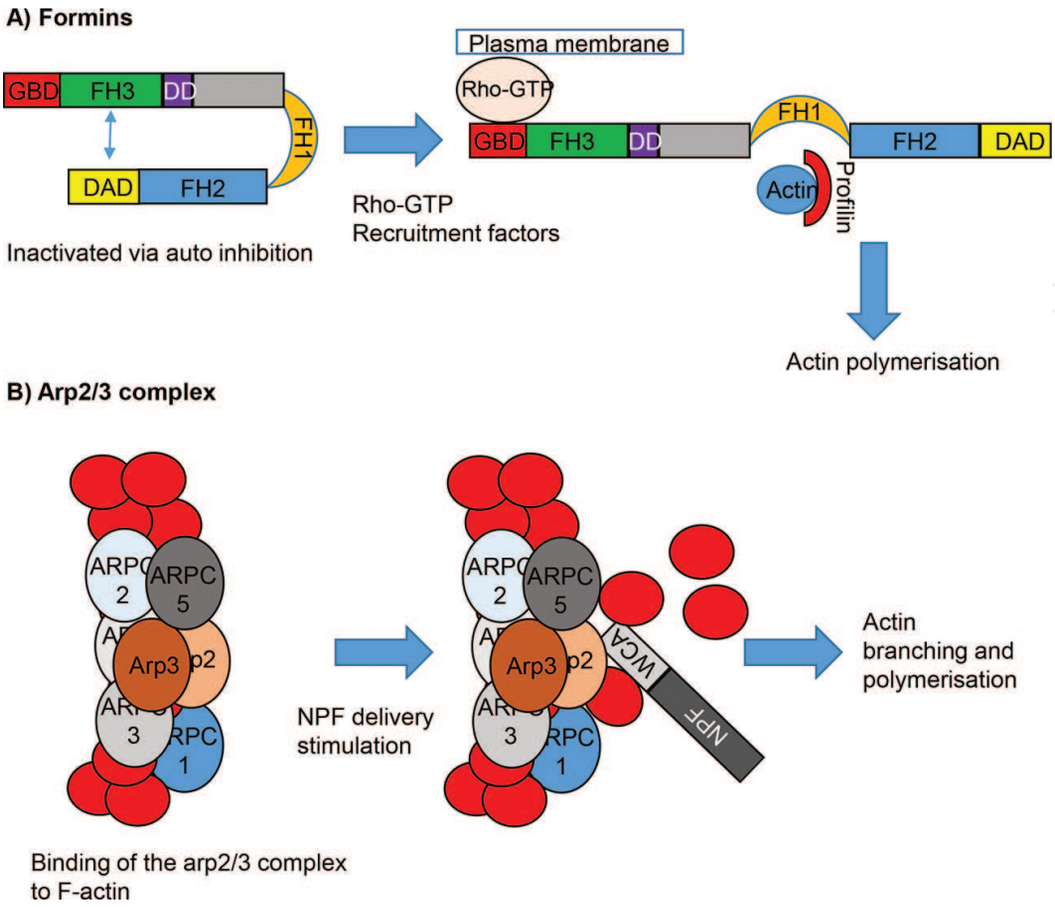

Figure 3. Cartoon showing some of the regulation steps for different actin nucleating proteins. (A) Activation of the Diaphanous-related formin. Autoinhibition of the actin nucleating ability is due to the interaction of the C-terminal Diaphanous auto-regulation domain (DAD) with the N-terminal FH3 (Formin homology) domain. Rho-GTP Binding of the GTP bound Rho to the GTPase binding domain (GBD) region is thought to lead to a partial displacement of the DAD as well as relocalisation of the complex, resulting in the unfolding of the protein and the relieve of the autoinhibition (DD dimerization domain). (B) Cartoon representation of the Arp2/3 complex and nucleation of a branched filament. The Arp2/3 complex initially binds to the pointed end of the mother F-actin. Binding of the WCA domain of a nucleation promoting factor (NPF) to exposed regions of Arp2 and Arp3 allows the delivery of actin monomer and initiate the polymerisation of a nascent branched filament as elegantly demonstrated [52].

the FH3 and DD (dimerization domain) regions on mDia also mediate its membrane localisation [45, 46], indicating that other proteins capable of interaction with such domains could be efficient regulators. The liprin family have been suggested to possess such properties and have been put forward as another series of proteins which may affect formin cellular functions [47].

Arp2/3 complex. Another regulator of actin nucleation and polymerisation that plays a critical role in the process of formation of lamellipodia and filopodia structures is the 220kDa Arp2/3 factor [48]. Composed of seven different subunits (ARPC1-5, Arp2 and Arp3), this complex promotes the formation of newly formed actin filament from the sides of existing filaments, forming a 70° side-branched network from pre-existing filaments [49, 50] (**Figure 3**). This property is predominantly the result of a striking similarity between the Arp2 and Arp3 proteins and that of monomeric actin molecules [51], providing a mimicking dimer that serves as a cooperative docking for other actin subunits and in doing so, accelerates the nucleation

process and thereby reduces the rate of the limiting step at this stage [52]. Whilst all components of this hetero-heptamer are critical for the generation of newly formed actin arrays from the pointed end, albeit with distinct functions, the Arp2 and Arp3 proteins are seen as the principal components responsible for establishing the initial base of the newly assembled filament [52]. The other components, especially ARPC1, are mainly involved in the binding to the mother filament [53, 54] (**Figure 3**). Interestingly, weak basal activity of the purified Arp2/3 complex in promoting actin nucleation and branch formation [55, 56] highlights its intrinsic association with other regulators [57]. Activation of the Arp2/3 complex is regulated by different complexes at distinct cellular locations. Whilst Arp2/3 is controlled by the WAVE regulatory complex in a Rac-GTPase pathway in lamellipodia, the Wiskott-Aldrich syndrome protein family (WASP), downstream of Cdc42, is predominantly implicated with the regulation of Arp2/3 in filopodia [58]. By all accounts, these nucleation-promoting factors (NPF) stimulate Arp2/3 mediated-nucleation through a WCA domain found at the C-terminus (**Figure 3**). It is thought that the WH2 region within the WCA domain is responsible for binding and therefore delivering the actin monomer, whilst the CA sequence promotes binding to the exposed regions of both Arp2 and Arp3 [59]. It is the clustering of the different subunits, along with the newly added actin molecule that encourages formation of a new nucleus and further actin polymerisation, resulting in the elongation of 70° side-branched network. Since the NPF family has been continuously expanding, it is now subcategorised into five groups including WASp and neural Wiskott-Aldrich syndrome protein (N-WASP), three SCAR/WAVE proteins and the recently identified factors WASH, WHAMM and JMY [60].

Taken all together, actin polymerization at the leading edge is a vital process for cellular migration, through the orchestrations of events that will ultimately lead to different cellular protrusion events. In this section, different actin polymerization factors and their functions (Arp2/3 and Formin) were briefly explored. One should remember that this is only a preferential view in regards to their potential involvements through a S100A4-dependent process and that numerous other regulators not mentioned here play equally vital roles in the process of actin remodelling and cellular migration.

Away from the leading edge and the protrusions of the lamellipodia and filopodia, the array of filamentous actin is seen to exist as more bundling rather than the branched sheets reported previously, mainly due to the interaction of different actin-binding proteins. This contractile network is seen as a unique structural complex, spatially posterior to the lamellipodium, and is referred to as the lamellum [61].

2.2. Lamellum and cellular contractions

In the spatial arrangement of the lamellum, filaments are organised in different structures, known as stress, dorsal and ventral fibres; they are the result of interaction of the actin filaments with different partners (**Figure 2**). It is in this context and primarily through the control of the tropomyosin and myosins that contractile forces are exerted to manipulate their overall organisation. Generation of such tensile forces is provided by the myosin network, mainly non-muscle myosin II (NMMII) which is responsible for the majority of the morphological and architectural reorganisations that promote cell movement.

Tropomyosin. The tropomyosin (tpm) family is composed of four separate genes, TPM1-4 which can be further subdivided, due to different alternative splicing and post-translational modifications, resulting in the presence of more than 40 tpm products [62, 63]. Interestingly, these isoforms have been shown to interact differentially with actin filaments, ensuing biophysical and dynamic property changes, as well as different subpopulations occurring in different locations and in abundance [64]. It is unclear today, how these association-promoting mechanisms are regulated over time and space, to result in such highly selective and discriminatory organisation [65, 66], but all interactions necessitate dimerization as well as head to tail contact between individual complexes to form continuous actin/tropomyosin filaments [67]. The formation of these highly selective complexes is thought to seclude, or at least regulate the interactions of other actin-binding proteins with these actin filaments, therefore playing a major role in determining the functions of different filaments [68, 69]. For instance, the absence of tropomyosin in the leading edge is thought to be a predominant factor that allows specific branching of the actin network, since different isoforms have been shown to compete and inhibit the actin polymerisation of Arp2/3, at least in vitro [52, 70]. Equally important is the fact that tropomyosin has been implicated in the regulation and recruitments of NMMII in stress fibre formation [68], regulating both elasticity and stiffness [71]

The overall organisation of the actin cytoskeleton can also be dictated by actin bundling and contractile motor proteins. Binding of individual filaments, actin cross-linking and motor proteins allow the formation of thicker, linear and either paralleled or antiparallel filamentous F-actin networks that can be found in all subcellular localisations. In the lamellum, the class II non-muscle myosin family has been shown to be a key regulator, participating in the bundling of actin filaments and generating mechanical forces, which result in filaments sliding and/or contractions [72, 73].

Non-muscle Myosin II family. The myosin II family, which encompasses a group of 34 different isoforms, are expressed in all eukaryotes, except plants with 15 genes corresponding to the myosin II cluster. These myosin II motor proteins are exclusively expressed in non-muscles cells and can therefore be referred to as non-muscle myosin II (NMMII). In its fully formed state, the NMMII complex corresponds to a 525 kDa structure composed of six non-covalently associated polypeptides. The backbone of this is a homodimeric myosin heavy chain containing a head domain and a long coiled-coil rod domain, separated by a neck area. Two essential light chains and two regulatory light chains bind to this backbone [74]. The N-terminal head portion of the heavy chain is globular in structure and possesses the actin-binding domains as well as the ATPase activity which is required for movement towards the plus end of the actin filament, thereby inducing sliding between filaments and force generation. In contrast, the long coiled-coil C-terminal part of this protein is essential for dimerization and further assembly of one hexamer to another thereby forming a multimeric network of bipolar NMMII with motor domains positioned at both ends of the filaments. Bipolar filaments of NMMII formation are the result of electrostatic interactions between these C-terminal helical tails [75] and are essential for its cellular functions. Stability of these NMMII filaments is controlled by phosphorylation of the myosin heavy chain [76, 77] or by interaction with proteins that recognize the C-terminal helical tail region.

http://dx.doi.org/10.5772/66940

invasion/metastasis [163, 164] will pave the way for the development of further drugs that can regulate S100A4 interaction with the actomyosin architecture.

Acknowledgements

I would like to apologise for the numerous studies, which have significantly improved our understanding of actin dependent processes regulating cellular motility and invasion, but could not be included in this work owing to limits on the number of references. Special thanks are given to Prof. Philip S. Rudland (the University of Liverpool) for his critical comments on the chapter and for Prof. David Poyner (Aston University) for his invaluable help with the preparation of the different actin structures used.

Author details

Stephane R. Gross

Address all correspondence to: S.R.Gross@aston.ac.uk

School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET, UK

References

[1] Nichols, J.M., D. Veltman, and R.R. Kay, *Chemotaxis of a model organism: progress with Dictyostelium*. Curr Opin Cell Biol, 2015. **36**: p. 7–12.

[2] Ridley, A.J., et al., *Cell migration: integrating signals from front to back*. Science, 2003. **302**(5651): p. 1704–9.

[3] Solnica-Krezel, L. and D.S. Sepich, *Gastrulation: making and shaping germ layers*. Annu Rev Cell Dev Biol, 2012. **28**: p. 687–717.

[4] Lambrechts, A., M. Van Troys, and C. Ampe, *The actin cytoskeleton in normal and pathological cell motility*. Int J Biochem Cell Biol, 2004. **36**(10): p. 1890–909.

[5] Ridley, A.J., *Rho GTPase signalling in cell migration*. Curr Opin Cell Biol, 2015. **36**: p. 103–12.

[6] Freeman, S.A. and S. Grinstein, *Phagocytosis: receptors, signal integration, and the cytoskeleton*. Immunol Rev, 2014. **262**(1): p. 193–215.

[7] Yang, H., et al., *Changes of cytoskeleton affect T cell biological behaviors*. Front Biosci (Landmark Ed), 2015. **20**: p. 829–37.

[8] Moulding, D.A., et al., *Actin cytoskeletal defects in immunodeficiency*. Immunol Rev, 2013. **256**(1): p. 282–99.

[9] Mayor, R. and S. Etienne-Manneville, *The front and rear of collective cell migration*. Nat Rev Mol Cell Biol, 2016. **17**(2): p. 97–109.

[10] Frugtniet, B., W.G. Jiang, and T.A. Martin, *Role of the WASP and WAVE family proteins in breast cancer invasion and metastasis*. Breast Cancer (Dove Med Press), 2015. **7**: p. 99–109.

[11] Fife, C.M., J.A. McCarroll, and M. Kavallaris, *Movers and shakers: cell cytoskeleton in cancer metastasis*. Br J Pharmacol, 2014. **171**(24): p. 5507–23.

[12] Friedl, P. and S. Alexander, *Cancer invasion and the microenvironment: plasticity and reciprocity*. Cell, 2011. **147**(5): p. 992–1009.

[13] Vicente-Manzanares, M. and A.R. Horwitz, *Cell migration: an overview*. Methods Mol Biol, 2011. **769**: p. 1–24.

[14] Newell-Litwa, K.A., R. Horwitz, and M.L. Lamers, *Non-muscle myosin II in disease: mechanisms and therapeutic opportunities*. Dis Model Mech, 2015. **8**(12): p. 1495–515.

[15] Gross, S.R., *Actin binding proteins: their ups and downs in metastatic life*. Cell Adh Migr, 2013. **7**(2): p. 199–213.

[16] Gross, S.R., et al., *Joining S100 proteins and migration: for better or for worse, in sickness and in health*. Cell Mol Life Sci, 2014. **71**(9): p. 1551–79.

[17] Davies, B.R., et al., *Induction of the metastatic phenotype by transfection of a benign rat mammary epithelial cell line with the gene for p9Ka, a rat calcium-binding protein, but not with the oncogene EJ-ras-1*. Oncogene, 1993. **8**(4): p. 999–1008.

[18] Mishra, S.K., H.R. Siddique, and M. Saleem, *S100A4 calcium-binding protein is key player in tumor progression and metastasis: preclinical and clinical evidence*. Cancer Metastasis Rev, 2012. **31**(1–2): p. 163–72.

[19] Yilmaz, M. and G. Christofori, *Mechanisms of motility in metastasizing cells*. Mol Cancer Res, 2010. **8**(5): p. 629–42.

[20] Bear, J.E. and J.M. Haugh, *Directed migration of mesenchymal cells: where signaling and the cytoskeleton meet*. Curr Opin Cell Biol, 2014. **30**: p. 74–82.

[21] Pollard, T.D. and G.G. Borisy, *Cellular motility driven by assembly and disassembly of actin filaments*. Cell, 2003. **112**(4): p. 453–65.

[22] Poincloux, R., et al., *Contractility of the cell rear drives invasion of breast tumor cells in 3D Matrigel*. Proc Natl Acad Sci USA, 2011. **108**(5): p. 1943–8.

[23] Ridley, A.J., *Life at the leading edge*. Cell, 2011. **145**(7): p. 1012–22.

[24] Le Clainche, C. and M.F. Carlier, *Regulation of actin assembly associated with protrusion and adhesion in cell migration*. Physiol Rev, 2008. **88**(2): p. 489–513.

[25] Gimona, M., et al., *Assembly and biological role of podosomes and invadopodia*. Curr Opin Cell Biol, 2008. **20**(2): p. 235–41.

[26] Kabsch, W., et al., *Atomic structure of the actin:DNase I complex*. Nature, 1990. **347**(6288): p. 37–44.

[27] Dominguez, R., *Actin-binding proteins—a unifying hypothesis*. Trends Biochem Sci, 2004. **29**(11): p. 572–8.

[28] dos Remedios, C.G., et al., *Actin binding proteins: regulation of cytoskeletal microfilaments*. Physiol Rev, 2003. **83**(2): p. 433–73.

[29] Kasai, M., S. Asakura, and F. Oosawa, *The cooperative nature of G-F transformation of actin*. Biochim Biophys Acta, 1962. **57**: p. 22–31.

[30] Kasai, M., S. Asakura, and F. Oosawa, *The G-F equilibrium in actin solutions under various conditions*. Biochim Biophys Acta, 1962. **57**: p. 13–21.

[31] Skau, C.T. and C.M. Waterman, Specification of architecture and function of actin structures by actin nucleation factors. Annu Rev Biophys, 2015. **44**: p. 285–310.

[32] Blanchoin, L., et al., *Actin dynamics, architecture, and mechanics in cell motility*. Physiol Rev, 2014. **94**(1): p. 235–63.

[33] Bugyi, B. and M.F. Carlier, *Control of actin filament treadmilling in cell motility*. Annu Rev Biophys, 2010. **39**: p. 449–70.

[34] Breitsprecher, D. and B.L. Goode, *Formins at a glance*. J Cell Sci, 2013. **126**(Pt 1): p. 1–7.

[35] Schonichen, A. and M. Geyer, *Fifteen formins for an actin filament: a molecular view on the regulation of human formins*. Biochim Biophys Acta, 2010. **1803**(2): p. 152–63.

[36] Thompson, M.E., et al., *FMNL3 FH2-actin structure gives insight into formin-mediated actin nucleation and elongation*. Nat Struct Mol Biol, 2013. **20**(1): p. 111–8.

[37] Baker, J.L., et al., *Electrostatic interactions between the Bni1p Formin FH2 domain and actin influence actin filament nucleation*. Structure, 2015. **23**(1): p. 68–79.

[38] Paul, A.S. and T.D. Pollard, *Review of the mechanism of processive actin filament elongation by formins*. Cell Motil Cytoskeleton, 2009. **66**(8): p. 606–17.

[39] Goode, B.L. and M.J. Eck, *Mechanism and function of formins in the control of actin assembly*. Annu Rev Biochem, 2007. **76**: p. 593–627.

[40] Kuhn, S. and M. Geyer, *Formins as effector proteins of Rho GTPases*. Small GTPases, 2014. **5**: p. e29513.

[41] Alberts, A.S., *Identification of a carboxyl-terminal diaphanous-related formin homology protein autoregulatory domain*. J Biol Chem, 2001. **276**(4): p. 2824–30.

[42] Bechtold, M., J. Schultz, and S. Bogdan, *FHOD proteins in actin dynamics—a formin' class of its own*. Small GTPases, 2014. **5**(2): p. 11.

[43] Rose, R., et al., *Structural and mechanistic insights into the interaction between Rho and mammalian Dia*. Nature, 2005. **435**(7041): p. 513–8.

[44] Lammers, M., et al., *Specificity of interactions between mDia isoforms and Rho proteins*. J Biol Chem, 2008. **283**(50): p. 35236–46.

[45] Kato, T., et al., *Localization of a mammalian homolog of diaphanous, mDia1, to the mitotic spindle in HeLa cells*. J Cell Sci, 2001. **114**(Pt 4): p. 775–84.

[46] Gorelik, R., et al., *Mechanisms of plasma membrane targeting of formin mDia2 through its amino terminal domains*. Mol Biol Cell, 2011. **22**(2): p. 189–201.

[47] Sakamoto, S., et al., *Liprin-alpha controls stress fiber formation by binding to mDia and regulating its membrane localization*. J Cell Sci, 2012. **125**(Pt 1): p. 108–20.

[48] Swaney, K.F. and R. Li, *Function and regulation of the Arp2/3 complex during cell migration in diverse environments*. Curr Opin Cell Biol, 2016. **42**: p. 63–72.

[49] Goley, E.D. and M.D. Welch, *The ARP2/3 complex: an actin nucleator comes of age*. Nat Rev Mol Cell Biol, 2006. **7**(10): p. 713–26.

[50] Volkmann, N., et al., *Structure of Arp2/3 complex in its activated state and in actin filament branch junctions*. Science, 2001. **293**(5539): p. 2456–9.

[51] Robinson, R.C., et al., *Crystal structure of Arp2/3 complex*. Science, 2001. **294**(5547): p. 1679–84.

[52] Rouiller, I., et al., *The structural basis of actin filament branching by the Arp2/3 complex*. J Cell Biol, 2008. **180**(5): p. 887–95.

[53] Insall, R.H. and L.M. Machesky, *Actin dynamics at the leading edge: from simple machinery to complex networks*. Dev Cell, 2009. **17**(3): p. 310–22.

[54] Beltzner, C.C. and T.D. Pollard, *Identification of functionally important residues of Arp2/3 complex by analysis of homology models from diverse species*. J Mol Biol, 2004. **336**(2): p. 551–65.

[55] Mullins, R.D., J.A. Heuser, and T.D. Pollard, *The interaction of Arp2/3 complex with actin: nucleation, high affinity pointed end capping, and formation of branching networks of filaments*. Proc Natl Acad Sci USA, 1998. **95**(11): p. 6181–6.

[56] Welch, M.D., et al., *Interaction of human Arp2/3 complex and the Listeria monocytogenes ActA protein in actin filament nucleation*. Science, 1998. **281**(5373): p. 105–8.

[57] Rodnick-Smith, M., et al., *Role and structural mechanism of WASP-triggered conformational changes in branched actin filament nucleation by Arp2/3 complex*. Proc Natl Acad Sci USA, 2016. **113**(27): p. E3834–43.

[58] Campellone, K.G. and M.D. Welch, *A nucleator arms race: cellular control of actin assembly*. Nat Rev Mol Cell Biol, 2010. **11**(4): p. 237–51.

[59] Firat-Karalar, E.N. and M.D. Welch, *New mechanisms and functions of actin nucleation*. Curr Opin Cell Biol, 2011. **23**(1): p. 4–13.

[60] Rottner, K., J. Hanisch, and K.G. Campellone, *WASH, WHAMM and JMY: regulation of Arp2/3 complex and beyond*. Trends Cell Biol, 2010. **20**(11): p. 650–61.

[61] Ponti, A., et al., *Two distinct actin networks drive the protrusion of migrating cells*. Science, 2004. **305**(5691): p. 1782–6.

[62] Colote, S., et al., *Evolution of tropomyosin functional domains: differential splicing and genomic constraints*. J Mol Evol, 1988. **27**(3): p. 228–35.

[63] Geeves, M.A., S.E. Hitchcock-DeGregori, and P.W. Gunning, *A systematic nomenclature for mammalian tropomyosin isoforms*. J Muscle Res Cell Motil, 2015. **36**(2): p. 147–53.

[64] Schevzov, G., et al., *Tropomyosin isoforms and reagents*. Bioarchitecture, 2011. **1**(4): p. 135–164.

[65] Gunning, P.W., et al., *Tropomyosin—master regulator of actin filament function in the cytoskeleton*. J Cell Sci, 2015. **128**(16): p. 2965–74.

[66] Johnson, M., D.A. East, and D.P. Mulvihill, *Formins determine the functional properties of actin filaments in yeast*. Curr Biol, 2014. **24**(13): p. 1525–30.

[67] Tobacman, L.S., *Cooperative binding of tropomyosin to actin*. Adv Exp Med Biol, 2008. **644**: p. 85–94.

[68] Bryce, N.S., et al., *Specification of actin filament function and molecular composition by tropomyosin isoforms*. Mol Biol Cell, 2003. **14**(3): p. 1002–16.

[69] Skau, C.T. and D.R. Kovar, *Fimbrin and tropomyosin competition regulates endocytosis and cytokinesis kinetics in fission yeast*. Curr Biol, 2010. **20**(16): p. 1415–22.

[70] Bugyi, B., D. Didry, and M.F. Carlier, *How tropomyosin regulates lamellipodial actin-based motility: a combined biochemical and reconstituted motility approach*. EMBO J, 2010. **29**(1): p. 14–26.

[71] Jalilian, I., et al., *Cell elasticity is regulated by the tropomyosin isoform composition of the actin cytoskeleton*. PLoS One, 2015. **10**(5): p. e0126214.

[72] Choi, C.K., et al., *Actin and alpha-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner*. Nat Cell Biol, 2008. **10**(9): p. 1039–50.

[73] Xu, X.S., et al., *During multicellular migration, myosin ii serves a structural role independent of its motor function*. Dev Biol, 2001. **232**(1): p. 255–64.

[74] Mooseker MS, F.B., The structural and functional diversity of the myosin family of actin-based molecular motors. In: Coluccio LM, editor (Netherlands) Springer, 2008: p. 1–34.

[75] Hostetter, D., et al., *Dictyostelium myosin bipolar thick filament formation: importance of charge and specific domains of the myosin rod*. PLoS Biol, 2004. **2**(11): p. e356.

[76] Dulyaninova, N.G., et al., *Regulation of myosin-IIA assembly and Mts1 binding by heavy chain phosphorylation*. Biochemistry, 2005. **44**(18): p. 6867–76.

[77] Clark, K., et al., *TRPM7 regulates myosin IIA filament stability and protein localization by heavy chain phosphorylation*. J Mol Biol, 2008. **378**(4): p. 790–803.

[78] Beach, J.R., et al., *Nonmuscle myosin II isoforms coassemble in living cells*. Curr Biol, 2014. **24**(10): p. 1160–6.

[79] Vicente-Manzanares, M., et al., *Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells*. J Cell Biol, 2007. **176**(5): p. 573–80.

[80] Burnette, D.T., et al., *A role for actin arcs in the leading-edge advance of migrating cells*. Nat Cell Biol, 2011. **13**(4): p. 371–81.

[81] Alexandrova, A.Y., et al., *Comparative dynamics of retrograde actin flow and focal adhesions: formation of nascent adhesions triggers transition from fast to slow flow*. PLoS One, 2008. **3**(9): p. e3234.

[82] Giannone, G., et al., *Lamellipodial actin mechanically links myosin activity with adhesion-site formation*. Cell, 2007. **128**(3): p. 561–75.

[83] Friedland, J.C., M.H. Lee, and D. Boettiger, *Mechanically activated integrin switch controls alpha5beta1 function*. Science, 2009. **323**(5914): p. 642–4.

[84] Zaidel-Bar, R., et al., *Functional atlas of the integrin adhesome*. Nat Cell Biol, 2007. **9**(8): p. 858–67.

[85] Jiang, G., et al., *Two-piconewton slip bond between fibronectin and the cytoskeleton depends on talin*. Nature, 2003. **424**(6946): p. 334–7.

[86] Aguilar-Cuenca, R., A. Juanes-Garcia, and M. Vicente-Manzanares, *Myosin II in mechanotransduction: master and commander of cell migration, morphogenesis, and cancer*. Cell Mol Life Sci, 2014. **71**(3): p. 479–92.

[87] Somlyo, A.P. and A.V. Somlyo, *Ca^{2+} sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase*. Physiol Rev, 2003. **83**(4): p. 1325–58.

[88] Adelstein, R.S. and M.A. Conti, *Phosphorylation of platelet myosin increases actin-activated myosin ATPase activity*. Nature, 1975. **256**(5518): p. 597–8.

[89] Betapudi, V., *Life without double-headed non-muscle myosin II motor proteins*. Front Chem, 2014. **2**: p. 45.

[90] Murakami, N., V.P. Chauhan, and M. Elzinga, *Two nonmuscle myosin II heavy chain isoforms expressed in rabbit brains: filament forming properties, the effects of phosphorylation by protein kinase C and casein kinase II, and location of the phosphorylation sites*. Biochemistry, 1998. **37**(7): p. 1989–2003.

[91] Liu, J., et al., *Refined model of the 10S conformation of smooth muscle myosin by cryo-electron microscopy 3D image reconstruction*. J Mol Biol, 2003. **329**(5): p. 963–72.

[92] Dulyaninova, N.G. and A.R. Bresnick, *The heavy chain has its day: regulation of myosin-II assembly*. Bioarchitecture, 2013. **3**(4): p. 77–85.

[93] Ronen, D. and S. Ravid, *Myosin II tailpiece determines its paracrystal structure, filament assembly properties, and cellular localization*. J Biol Chem, 2009. **284**(37): p. 24948–57.

[94] Nakasawa, T., et al., *Critical regions for assembly of vertebrate nonmuscle myosin II*. Biochemistry, 2005. **44**(1): p. 174–83.

[111] Sakamoto, S., S. Narumiya, and T. Ishizaki, *A new role of multi scaffold protein Liprin-alpha: liprin-alpha suppresses Rho-mDia mediated stress fiber formation*. Bioarchitecture, 2012. **2**(2): p. 43–49.

[112] Shen, J.C., et al., *Inhibitor of growth 4 suppresses cell spreading and cell migration by interacting with a novel binding partner, liprin alpha1*. Cancer Res, 2007. **67**(6): p. 2552–8.

[113] Asperti, C., et al., *Liprin-alpha1 promotes cell spreading on the extracellular matrix by affecting the distribution of activated integrins*. J Cell Sci, 2009. **122**(Pt 18): p. 3225–32.

[114] Astro, V., et al., *Liprin-alpha1, ERC1 and LL5 define polarized and dynamic structures that are implicated in cell migration*. J Cell Sci, 2014. **127**(Pt 17): p. 3862–76.

[115] Burridge, K. and K. Wennerberg, *Rho and Rac take center stage*. Cell, 2004. **116**(2): p. 167–79.

[116] Narumiya, S., M. Tanji, and T. Ishizaki, *Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion*. Cancer Metastasis Rev, 2009. **28**(1–2): p. 65–76.

[117] Tsuji, T., et al., *ROCK and mDia1 antagonize in Rho-dependent Rac activation in Swiss 3T3 fibroblasts*. J Cell Biol, 2002. **157**(5): p. 819–30.

[118] Pertz, O., et al., *Spatiotemporal dynamics of RhoA activity in migrating cells*. Nature, 2006. **440**(7087): p. 1069–72.

[119] O'Connor, K.L., B.K. Nguyen, and A.M. Mercurio, *RhoA function in lamellae formation and migration is regulated by the alpha6beta4 integrin and cAMP metabolism*. J Cell Biol, 2000. **148**(2): p. 253–8.

[120] Machacek, M., et al., *Coordination of Rho GTPase activities during cell protrusion*. Nature, 2009. **461**(7260): p. 99–103.

[121] Kurokawa, K. and M. Matsuda, *Localized RhoA activation as a requirement for the induction of membrane ruffling*. Mol Biol Cell, 2005. **16**(9): p. 4294–303.

[122] Chen, M., A.R. Bresnick, and K.L. O'Connor, *Coupling S100A4 to Rhotekin alters Rho signaling output in breast cancer cells*. Oncogene, 2013. **32**(32): p. 3754–64.

[123] Li, Z.H., et al., *Mts1 regulates the assembly of nonmuscle myosin-IIA*. Biochemistry, 2003. **42**(48): p. 14258–66.

[124] Ford, H.L., et al., *Effect of Mts1 on the structure and activity of nonmuscle myosin II*. Biochemistry, 1997. **36**(51): p. 16321–7.

[125] Ramagopal, U.A., et al., *Structure of the S100A4/myosin-IIA complex*. BMC Struct Biol, 2013. **13**: p. 31.

[126] Kiss, B., et al., *Structural determinants governing S100A4-induced isoform-selective disassembly of nonmuscle myosin II filaments*. FEBS J, 2016. **283**(11): p. 2164–80.

[127] Kiss, B., et al., *Crystal structure of the S100A4-nonmuscle myosin IIA tail fragment complex reveals an asymmetric target binding mechanism*. Proc Natl Acad Sci USA, 2012. **109**(16): p. 6048–53.

[128] Elliott, P.R., et al., *Asymmetric mode of Ca(2)(+)-S100A4 interaction with nonmuscle myosin IIA generates nanomolar affinity required for filament remodeling*. Structure, 2012. **20**(4): p. 654–66.

[129] Badyal, S.K., et al., *Mechanism of the Ca(2)+-dependent interaction between S100A4 and tail fragments of nonmuscle myosin heavy chain IIA*. J Mol Biol, 2011. **405**(4): p. 1004–26.

[130] Cai, Y., et al., *Nonmuscle myosin IIA-dependent force inhibits cell spreading and drives F-actin flow*. Biophys J, 2006. **91**(10): p. 3907–20.

[131] Even-Ram, S., et al., *Myosin IIA regulates cell motility and actomyosin-microtubule crosstalk*. Nat Cell Biol, 2007. **9**(3): p. 299–309.

[132] Lim, J.I., et al., *Protrusion and actin assembly are coupled to the organization of lamellar contractile structures*. Exp Cell Res, 2010. **316**(13): p. 2027–41.

[133] Shih, W. and S. Yamada, *Myosin IIA dependent retrograde flow drives 3D cell migration*. Biophys J, 2010. **98**(8): p. L29–31.

[134] Astin, J.W., et al., *Competition amongst Eph receptors regulates contact inhibition of locomotion and invasiveness in prostate cancer cells*. Nat Cell Biol, 2010. **12**(12): p. 1194–204.

[135] Li, Z.H., et al., *S100A4 regulates macrophage chemotaxis*. Mol Biol Cell, 2010. **21**(15): p. 2598–610.

[136] Takenaga, K., Y. Nakamura, and S. Sakiyama, *Cellular localization of pEL98 protein, an S100-related calcium binding protein, in fibroblasts and its tissue distribution analyzed by monoclonal antibodies*. Cell Struct Funct, 1994. **19**(3): p. 133–41.

[137] Takenaga, K., et al., *Binding of pEL98 protein, an S100-related calcium-binding protein, to nonmuscle tropomyosin*. J Cell Biol, 1994. **124**(5): p. 757–68.

[138] Chen, H., et al., *Binding to intracellular targets of the metastasis-inducing protein, S100A4 (p9Ka)*. Biochem Biophys Res Commun, 2001. **286**(5): p. 1212–7.

[139] Pasapera, A.M., et al., *Myosin II activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin phosphorylation*. J Cell Biol, 2010. **188**(6): p. 877–90.

[140] Dulyaninova, N.G., et al., *Myosin-IIA heavy-chain phosphorylation regulates the motility of MDA-MB-231 carcinoma cells*. Mol Biol Cell, 2007. **18**(8): p. 3144–55.

[141] Du, M., et al., *S100P dissociates myosin IIA filaments and focal adhesion sites to reduce cell adhesion and enhance cell migration*. J Biol Chem, 2012. **287**(19): p. 15330–44.

[142] Dahan, I., et al., *The tumor suppressor Lgl1 regulates NMII-A cellular distribution and focal adhesion morphology to optimize cell migration*. Mol Biol Cell, 2012. **23**(4): p. 591–601.

[143] Dahan, I., et al., *The tumor suppressor Lgl1 forms discrete complexes with NMII-A and Par6alpha-aPKCzeta that are affected by Lgl1 phosphorylation*. J Cell Sci, 2014. **127**(Pt 2): p. 295–304.

[144] van Wijk, E., et al., *A mutation in the gamma actin 1 (ACTG1) gene causes autosomal dominant hearing loss (DFNA20/26)*. J Med Genet, 2003. **40**(12): p. 879–84.

[145] Agaisse, H., *Molecular and cellular mechanisms of Shigella flexneri dissemination*. Front Cell Infect Microbiol, 2016. **6**: p. 29.

[146] Imai, K., S. Nonoyama, and H.D. Ochs, *WASP (Wiskott-Aldrich syndrome protein) gene mutations and phenotype*. Curr Opin Allergy Clin Immunol, 2003. **3**(6): p. 427–36.

[147] Lynch, E.D., et al., *Nonsyndromic deafness DFNA1 associated with mutation of a human homolog of the Drosophila gene diaphanous*. Science, 1997. **278**(5341): p. 1315–8.

[148] DeWard, A.D., et al., *The role of formins in human disease*. Biochim Biophys Acta, 2010. **1803**(2): p. 226–33.

[149] Randall, T.S. and E. Ehler, *A formin-g role during development and disease*. Eur J Cell Biol, 2014. **93**(5–6): p. 205–11.

[150] Zhang, K., et al., *S100A4 regulates motility and invasiveness of human esophageal squamous cell carcinoma through modulating the AKT/Slug signal pathway*. Dis Esophagus, 2012. **25**(8): p. 731–9.

[151] Sack, U., et al., *S100A4-induced cell motility and metastasis is restricted by the Wnt/beta-catenin pathway inhibitor calcimycin in colon cancer cells*. Mol Biol Cell, 2011. **22**(18): p. 3344–54.

[152] Wang, H., et al., *Activation of the PI3K/Akt/mTOR/p70S6K pathway is involved in S100A4-induced viability and migration in colorectal cancer cells*. Int J Med Sci, 2014. **11**(8): p. 841–9.

[153] Zhang, J., et al., *S100A4 regulates migration and invasion in hepatocellular carcinoma HepG2 cells via NF-kappaB-dependent MMP-9 signal*. Eur Rev Med Pharmacol Sci, 2013. **17**(17): p. 2372–82.

[154] Malashkevich, V.N., et al., *Structure of Ca^{2+}-bound S100A4 and its interaction with peptides derived from nonmuscle myosin-IIA*. Biochemistry, 2008. **47**(18): p. 5111–26.

[155] Forst, B., et al., *Metastasis-inducing S100A4 and RANTES cooperate in promoting tumor progression in mice*. PLoS One, 2010. **5**(4): p. e10374.

[156] Semov, A., et al., *Metastasis-associated protein S100A4 induces angiogenesis through interaction with Annexin II and accelerated plasmin formation*. J Biol Chem, 2005. **280**(21): p. 20833–41.

[157] Cabezon, T., et al., *Expression of S100A4 by a variety of cell types present in the tumor microenvironment of human breast cancer*. Int J Cancer, 2007. **121**(7): p. 1433–44.

[158] Ambartsumian, N., et al., *The metastasis-associated Mts1(S100A4) protein could act as an angiogenic factor*. Oncogene, 2001. **20**(34): p. 4685–95.

[159] Schmidt-Hansen, B., et al., *Extracellular S100A4(mts1) stimulates invasive growth of mouse endothelial cells and modulates MMP-13 matrix metalloproteinase activity*. Oncogene, 2004. **23**(32): p. 5487–95.

[160] Spiekerkoetter, E., et al., *S100A4 and bone morphogenetic protein-2 codependently induce vascular smooth muscle cell migration via phospho-extracellular signal-regulated kinase and chloride intracellular channel 4*. Circ Res, 2009. **105**(7): p. 639–47, 13 p following 647.

[161] Garrett, S.C., et al., *A biosensor of S100A4 metastasis factor activation: inhibitor screening and cellular activation dynamics*. Biochemistry, 2008. **47**(3): p. 986–96.

[162] Malashkevich, V.N., et al., *Phenothiazines inhibit S100A4 function by inducing protein oligomerization*. Proc Natl Acad Sci USA, 2010. **107**(19): p. 8605–10.

[163] Klingelhofer, J., et al., *Anti-S100A4 antibody suppresses metastasis formation by blocking stroma cell invasion*. Neoplasia, 2012. **14**(12): p. 1260–8.

[164] Sack, U., et al., *Novel effect of antihelminthic Niclosamide on S100A4-mediated metastatic progression in colon cancer*. J Natl Cancer Inst, 2011. **103**(13): p. 1018–36.

[165] Otterbein, L.R., P. Graceffa, and R. Dominguez, *The crystal structure of uncomplexed actin in the ADP state*. Science, 2001. **293**(5530): p. 708–11.

[166] Holmes, K.C., et al., *Atomic model of the actin filament*. Nature, 1990. **347**(6288): p. 44–9.